

Micropropagation of *Santalum Album* L. (Sandalwood)

S. Aghi Zion Inbakani¹, S. Sathishkumar², Bakan Jagdish Sudhakar³

¹Technical Assistant, ²Research Scholar, ³Deputy Conservator of Forests,
^{1,2,3}State Forest Research Institute, Kolapakkam, Chennai, Tamil Nadu, India

ABSTRACT

An efficient plant regeneration protocol was developed for *Santalum album* L. (Santalaceae), an economically important species. Plant regeneration was achieved using nodal explants and leaf disc on Murashige and Skoog (MS) medium for direct shoot regeneration. Effect of Plant Growth Regulators (PGR) like 6-Benzyl Adenine (BA), Kinetin (KN) and 2-Isopentenyl adenine (2-iP) on shoot initiation; 2-Isopentenyl adenine and Gibberellic acid (GA₃) for shoot elongation and multiple shoot formation and Indole-3-Butyric Acid (IBA) and α -Naphthalene Acetic Acid (NAA) for rooting was studied. Among the explants tested for shoot induction, nodal segments proved good results. The best treatment for obtaining shoot induction was 3.0mg/L BAP and for rooting 1 mg/L of IBA was found to be the best treatment combination for maximum sprouting of shoot and rooting. After six the rooted plantlets were transferred for hardening, 20% of plantlets survived and resumed growth in the mixture of soil, vermiculite and sand (1:1:1).

KEYWORDS: *Santalum album*; MS media; PGR; Zip; hardening

How to cite this paper: S. Aghi Zion Inbakani | S. Sathishkumar | Bakan Jagdish Sudhakar "Micropropagation of *Santalum Album* L. (Sandalwood)" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-4, June 2021, pp.1720-1722, URL: www.ijtsrd.com/papers/ijtsrd43698.pdf



IJTSRD43698

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INTRODUCTION

Santalum album L. belongs to the santalaceae family, a medium-sized evergreen hemi root parasitic tree. Sandal is considered as one of the most valuable tree in the world. The sandal is commercially and culturally valued for its oil that is used in perfumes, cosmetics, and also in Agarbathi (incense sticks) industries. *Santalum album* has the highest oil content (about 6%) of the species of the genus *Santalum*. The sandalwood and its oil demand (80%-90%) in the international market have been fulfilled by Indian sandalwood for decades. Production of Sandalwood has fallen sharply over the past decades due to the mushrooming of illegal sandalwood processing units. Conventional methods of asexual propagation like grafting, budding, layering etc. for many of the plants and trees are often too slow or fail completely.

In vitro regeneration techniques can be used to encounter difficulties of traditional vegetative propagation methods of superior clones. *In vitro* propagation of sandalwood was attempted as early as 1963. Induction of callus from mature endosperm on modified White's medium was reported, but the callus did not proliferate further (Rangaswamy and Rao, 1963). Various explants such as embryo, hypocotyls, shoot tip, nodal segment and cell suspension cultures with varying degree of success have been used for rapid multiplication of sandal trees. There is only one published report on shoot bud formation directly from *in vitro* cultured leaves for sandal (Mujib, 2005). In woody species relatively little information is available on shoots formed directly on leaves without a callus phase (Preece *et al.*, 1993). Leaves obtained

from *in vitro* grown plants provide a useful source of explants which eliminate the risk of contamination. Micropropagation using tissue culture allows much greater control and manipulation of the development of tissues within the culture tube than conventional methods. However, a systematic study on the effects of combinations of plant growth regulators on morphogenesis is still insufficient, which may overlook the potential combinations of certain plant growth regulators that are more suitable. The aim of the present study is to develop a potential system of *in vitro* regeneration of the highly valued tree *Santalum album*.

MATERIALS AND METHOD

The study was carried out in the tissue culture laboratory of State Forest Research Institute, kolapakkam, Chennai during the period 2017 to 2018. Nodal segments and leaves were collected from selected *Santalum album* L. germplasm of SFRI, Kolapakkam. The explants (nodes) of 2.0 – 2.5 cm in length and first and second leaves of tender shoots were dissected out from the mother plant and collected in sterile distilled water and brought to the lab. For this purpose, the explants were shaken gently with water for 30 minutes in a conical flask. A slant cut was given at the base of the nodal explants to expose more surface area for better transport of nutrients. The explants were washed thoroughly with running tap water and then disinfected with Tween 20 solution (0.1%) for 5-10 minutes and washed thoroughly with running tap water. After an hour, the explants were treated with 1% Bavistin® (BASF, Germany) for 10 minutes

in horizontal shaker at 30 rpm and surface sterilized in 0.1% HgCl₂ for 1 min. The explants were then treated with 70% (v/v) ethanol for 1 minute followed by a treatment of aqueous solution of and finally the explants were rinsed (3 times) in sterilized water to remove the sterilants under sterilized condition.

Preparation of media for culturing

All stock solutions and final medium were prepared by following the procedure of Bhojwani and Razdan (1983). The prepared MS (Murashige and Skoog, 1962) medium was sterilized at 121°C (15 lbs pressure) for 20 minutes which was fortified with 30% sucrose, 0.8% Agar and various combinations of Plant Growth Hormones and adjuvants.

Screening of explants and basal medium

The explants were inoculated in MS basal medium supplemented with various concentrations and combinations of BAP and 2ip. The basal MS medium served as control. The following were the different media combinations per litre used in this study. i) MS (basal), ii) MS + BAP (1mg) + 2ip (10mg), iii) MS + BAP (2mg) + 2ip (20mg), iv) MS + BAP (3mg) + 2ip (30mg), v) MS + BAP (4mg) + 2ip (40mg) and vi) MS + BAP (5mg) + 2ip (50mg). The cultures were exposed to a light (approximately 2000 lux) and darkness cycle of 16 hours and 8 hours respectively. The culture room temperature was maintained at 25 ± 2°C. The following observations were made. i) Time taken for shoot initiation, ii) Percentage of shoot initiated and iii) length of shoots.

Screening culture medium for root induction

Individual shoots of 4.0-4.5 cm length were dissected out from the multiple shoots aseptically and cultured on half strength MS medium containing various concentrations of auxins-IBA in order to induce rhizogenesis. The following concentrations per litre were used in this study. i) MS (basal), ii) MS + IBA (0.5mg), iii) IBA (1mg), iv) IBA (1.5mg) v) IBA (2mg) and vi) IBA (2.5mg). The cultures were exposed to similar conditions extended for shoot induction. The following observations were recorded. i) Percent cultures with root induction, ii) Root length.

Hardening

For *ex vitro* establishment, well rooted plantlets were rinsed thoroughly with sterile water to remove residual nutrient from the plant body and transplanted to paper cups containing a mixture of red soil, vermicast and sand in a ratio of 1:1:1. The rooted plantlets were transferred to green house under 60-70% humidity. The plantlets were fertigated with dilute MS basal media. After 15 days, the fully acclimatized plantlets were transferred to pots (6 cm dia) containing red soil, vermiculite and sand in 1:1:1 ratio. Established plantlets were then transferred to bigger pots (14 cm dia).

RESULT AND DISCUSSION

Sexual reproduction by seed is not trustworthy since it may result in high degree of heterogeneity among plants. Traditional breeding methods are overwhelming and expensive particularly in tree species, due to long gestation period. Therefore, propagation of plants through tissue culture is gaining fame in forestry to reproduce plant taxa otherwise tricky to propagate conventionally or by vegetative means (Govil and Gupta, 1997). Tissue culture plays an important role in solving the problem through rapid *in vitro* multiplication of novel genotypes and to screen useful variants.

Morphological Response (Shoot & Root)

In the present study, among different explants (nodal and leaf disc) the highest morphogenic response was recorded by nodal explants (50%) which differed by leaf explants (10%).

Standardizing suitable medium for shoot induction Effect of BAP and 2ip on multiple shoot induction

Among the various treatments, the treatment MS + BAP (3mg) + 2ip (30mg) (50.75 %) followed by MS + BAP (2mg) + 2ip (20mg) (50.25 %) recorded higher per cent shoot induction compared to MS basal medium (14.50 %). All other treatments showed increased shoot induction of 38.85% (MS + BAP (1mg) + 2ip (10mg), MS + BAP (4mg) + 2ip (40mg) and MS + BAP (5mg) + 2ip (50mg) 40.25 % (MS + 1.0 mg/l-1 BAP) over that of MS basal medium (14.50 %). The two treatment viz., MS + BAP (3mg) + 2ip (30mg) (50%) and MS + BAP (2mg) + 2ip (20mg) (45%) recorded higher number of percentage of shoots initiated over MS basal medium (16%). The average shoot length was 3.56, however the treatment MS + 3.0 mg/l-1 BAP (5.08 cm) proved superior but on par with MS + 4.0 mg/l-1 BAP (4.25 cm) (Table 1, Figure 1 - 3). Thus, from the overall perspective the treatment MS + 3.0 mg/l-1 BAP was brought into sharp focus for shoot multiplication. Micro propagation studies in *Santalum album* L. indicated that cytokinins-kinetin is the best treatment for shoot induction (Kalpataru Dutta Mudoj and Mina Borthakaur, 2009). But in this study, an adjuvant 2ip was used instead of cytokinins. The shoot bud induction was observed and it was as same as in the earlier study using cytokinins.

Effect of IBA on root induction

The different concentration of auxins-IBA have recorded significantly improved results towards percent culture with root induction and the length of roots compared to the MS basal medium which has not recorded any root induction. Among the treatments the MS medium supplemented with MS + 2.0 mg of IBA recorded higher root induction (65%) followed by MS medium supplemented with MS + 1.5 mg of IBA (55.20 %) (Table 2; Figure 4 & 5). Kalpataru Dutta Mudoj and Mina Borthakur (2009) reported the maximum root induction in MS + BAP 1.0 mg l⁻¹ + NAA 3.0 mg l⁻¹. Auxins were known to induce rooting in many woody plants (Bhatnagar et al., 1968). The positive effect of IBA on rooting of *in vitro* propagated plants had been established in many tree species viz., *Prosopis tamaruga*, *Albizia procera*, *teak*, *Sterculia urens* and *Zizyphus mauritiana* which are consistent with the results of present findings.

Complete regeneration of shoots, since they gave rise to complete plantlets after rhizogenesis on rooting medium. Rooted plantlets were elongated and transferred (Fig. 6) for hardening in Green House. Later these regenerants were transferred to the Net House or Nursery for 25- 30 days (Fig. 7) for acclimatization. The plants, after survival in the net house conditions, were evaluated visually on the basis of their appearance. Although the traits were not scored quantitatively, regenerated plants were found phenotypically normal and true to the type.

Conclusion

The results showed the ability of the nodal explants to produce higher number of shootlets without any intervening callus phase, where all the plantlets were uniform in height and growth. Hence, we propose this protocol a simple, economical, rapid and highly

reproducible to obtain more plantlets within a short period of time.



Fig. 1: Shoot Induction



Fig. 2: Multiple Shoot Induction



Fig. 3: Well grown shoots



Fig. 4: Sub-cultured shoot for rooting



Fig. 5: Invitro rooting



Fig. 6: Hardening in green house



Fig. 7: Hardening in net house/nursery

TABLE 1: INVITRO RESPONSE OF SHOOT

Concentration of growth regulators	Time of shoot initiated (days)	Percentage of shoot initiated (%)	Length of shoot (cm)
MS (basal)	22	25	2.29
MS + BAP (1mg) + 2ip (10mg)	10	40	4.30
MS + BAP (2mg) + 2ip (20mg)	9	45	4.44
MS + BAP (3mg) + 2ip (30mg)	7	50	5.08
MS + BAP (4mg) + 2ip (40mg)	15	40	4.27
MS + BAP (5mg) + 2ip (50mg)	17	40	4.27

TABLE 2: INVITRO RESPONSE OF ROOT

Concentration of growth regulators	Percentage of shoot induction (%)	Length of root (cm)
MS (basal)	0	0
MS + IBA (0.5mg)	27.30	0.52
MS + IBA (1mg)	42	1.24
MS + IBA (1.5mg)	55.20	2.19
MS + IBA (2mg)	65	4.72
MS + IBA (2.5mg)	37	1.09

Acknowledgment

We owe a deep sense of gratitude to State Forest Research Institute, Chennai of Tamil Nadu Forest Department for their esteemed financial assistance. Overwhelmed with emotions we would like to thank Thiru. K. Sathyamoorthy IFS, Dr. P. Durairasu IFS, Dr. V. Naganathan IFS, Thiru. K. Chandrasekaran FRO, Thiru. A. Anil Kumar Forester and Dr. Florida Tilton who gave us an eternal feeling to do this work. Finally, we thank Dr. H. Dileep Kumar IFS for his unconditional support.

References

- [1] A. Mujib, "In vitro regeneration of sandal (*Santalum album* L.) from leaves", *Turk J Bot*, 2005, vol. 29, pp. 63-67.
- [2] J.E. Preece, M.R. Imel, and A. Shevade, "Regeneration of Rhododendron PJM group plants from leaf explants", *Journal of the American Rhododendron Society*, 1993, vol. 47, pp. 68-71.
- [3] Kalapataru Dutta Mudoi and Mina Borthakur. *In vitro* propagation of *Bambusa balcooa* through nodal explants from field- grown culms and scope for upscaling, *Curr. Sci.* 2009, vol. 96(7), pp. 962 -966.
- [4] S Govil and SC Gupta, Commercialization of plant tissue culture in India, *Plant Cell, Tissue Organ Culture*, 1997, vol. 51, pp. 65-73.